

Claims:

1. An arrangement for visualizing molecules, movements thereof, and interactions between molecules, and molecular processes in a sample, in particular molecules and processes in biological cells, by using the single dye tracing (SDT) method, comprising

- at least one source of light for large-area fluorescence excitation via single or multi-photon absorption by equal or different marker molecules to molecules in the sample
- a sample holding means for accommodating the sample,
- a highly-sensitive detection and analysis system comprising a charged coupled device (CCD) camera, the sample or the sample holding means, respectively, and/or the detection and analysis system being shiftable relative to each other during the measuring process, and
- a control unit for coordinating and synchronizing illumination times and, optionally, wave lengths of the lateral or vertical movement of the sample or of the sample holding means, respectively, with the sample as well as, optionally, the positioning and shifting of the images of each sample position of the pixel array of the CCD camera.

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2. An arrangement according to claim 1, characterized in that at least one source of light is a laser, in particular an acousto-optically switchable laser light.

3. An arrangement according to claim 1 or 2, characterized in that the source of light is an argon laser, a dye laser and/or a two-photon fluorescence excitation laser.

4. An arrangement according to any one of claims 1 to 3, characterized in that the control unit comprises a pulse transmitter and a software for controlling the source(s) of light and the movement of the sample.

5. An arrangement according to any one of claims 1 to 4, characterized in that the CCD camera comprises a frame shift mode and a continuous readout mode.

6. An arrangement according to any one of claims 1 to 5, characterized in that it comprises an epifluorescence microscope, preferably with a collecting efficiency of fluorescence quanta of  $> 3\%$ , at 40- to 100-fold magnification.

7. An arrangement according to any one of claims 1 to 6, characterized in that the CCD camera

- is  $N_2$ -cooled,

- comprises a large pixel array, in particular a pixel array  $\geq 1340 \times 1300$ ,
- comprises a conversion of photons into electrons of from 0.8 to 0.9 in the optical range,
- has a readout noise of only a few electrons per pixel at 1  $\mu$ s/pixel readout speed,
- comprises  $\ll 1$  dark counts/pixel  $\times$  s, and/or
- comprises a line shift rate of  $> 3 \times 10^5$ /s.

8. An arrangement according to any one of claims 1 to 7, characterized in that the sample comprises a molecule library prepared by combinatorial chemistry.

9. An arrangement according to any one of claims 1 to 8, characterized in that the sample comprises a multi-well plate or a micro (nano) titer plate.

10. An arrangement according to any one of claims 1 to 9, characterized in that the sample carrying means is a flowthrough cell.

11. An arrangement according to any one of claims 1 to 10, characterized in that the focussing plane of the detection and analysis system is shiftable step-wise along the z-direction by a piezo element.

12. An arrangement according to any one of claims 1

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to 11, characterized in that it comprises an epifluorescence microscope with a parallel beam region as the light source, which includes a galvano-optical mirror in the parallel beam region.

13. A method for visualizing molecules, movements thereof, and interactions between molecules, and molecular processes in a sample, in particular molecules and processes in biological cells, by using the single dye tracing (SDT) method, characterized in that a sample in which certain molecules have been labeled with marker molecules is introduced into an arrangement according to any one of claims 1 to 12, that the sample is imaged by the CCD camera on a pixel array, wherein the sample and/or the detection and analysis system are shifted relative to each other by using the frame shift of the CCD camera, so that the signals of each individual molecule in the sample are collected in the same pixels after conversion into electrons until the single molecule signal exceeds a certain minimum signal/noise ratio.

14. A method according to claim 13, characterized in that the relative movement of the sample is controlled corresponding to the frame shift of the CCD camera.

15. A method according to claim 13 or 14, character-

ized in that the relative movement of the sample in lateral direction is constant and continuous.

16. A method for quasi-simultaneous imaging of fluorescence-labeled molecules in their distribution over entire biological cells and for observing molecular movements and processes by repeating this imaging at temporal intervals by using the SDT method, characterized in that a sample in which certain molecules have been labeled with marker molecules is introduced into an arrangement according to any one of claims 1 to 12, the fluorescence image for one focussing plane is imaged on the pixel array of the CCD camera, the focussing plane is shifted step-wise along the z-direction by a piezo element, wherein the fluorescence images for each plane are separately arranged on the pixel array, and after imaging of all the focussing planes, the image of the fluorescence-labeled molecules in the cells is calculated, whereupon, optionally, imaging of the focussing planes is repeated so as to trace molecular movements and processes by consecutively arranging images of all the focussing planes.

17. A method according to any one of claims 13 to 16, characterized in that the images on the pixel array of the CCD camera are captured at a rate of from 1 to 3 ms per image and with a capacity of up to 300 images per

array, with an image size of 80 x 80 pixels.

18. A method according to any one of claims 13 to 17, characterized in that at least two different types of molecules in the sample are labelled with at least two different fluorescence markers.

19. A method according to any one of claims 13 to 18, characterized in that the fluorescence imaging is effected for two orthogonal polarization directions for each fluorescence marker, preferably by dividing the image into two images with orthogonal polarization direction, by using a Wollaston prism and a source of light which comprises a parallel beam region, wherein the Wollaston prism is used in the parallel beam region of the source of light.

20. A method according to any one of claims 13 to 19, characterized in that the sample comprises cells with low autofluorescence.

21. A method according to any one of claims 13 to 20, characterized in that the method is carried out as a high throughput analysis.

22. A method according to any one of claims 13 to 21, characterized in that as the sample, a molecule library

is analyzed, preferably a molecule library prepared by combinatorial chemistry.

23. A method according to any one of claims 13 to 22, characterized in that the interaction of a molecule library with biological cells is analyzed.

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